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PCT/PTO 29 JAN 2000

(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT &amp; TRADEMARK OFFICE

**TRANSMITTAL LETTER TO THE  
UNITED STATES  
DESIGNATED/ELECTED OFFICE  
(DO/EO/US) CONCERNING A FILING  
UNDER 35 U.S.C. 371**

ATTORNEY'S DOCKET NUMBER  
108366U.S. APPLICATION NO.  
(if known, sec 37 C.F.R.1.5)**09/744679**INTERNATIONAL APPLICATION NO.  
PCT/IL99/00079INTERNATIONAL FILING DATE  
February 5, 1999PRIORITY DATE CLAIMED  
August 7, 1998TITLE OF INVENTION  
METHOD FOR TREATMENT OF INVASIVE CELLSAPPLICANT FOR DO/EO/US  
Rachel BAR-SHAVIT

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

**Items 11. to 16. below concern other document(s) or information included:**

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.  
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☒ Entitlement to small entity status is hereby asserted.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) <b>09/744679</b>		INTERNATIONAL APPLICATION NO. PCT/IL99/00079		ATTORNEY'S DOCKET NUMBER 108366	
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17. <input checked="" type="checkbox"/> The following fees are submitted:  <b>Basic National fee (37 CFR 1.492(a)(1)-(5)):</b>  Search Report has been prepared by the EPO or JPO ....\$860.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) .....\$690.00  No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$710.00  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....\$1,000.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....\$ 100.00  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				CALCULATIONS		PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$			
Claims	Number Filed	Number Extra	Rate				
Total Claims	19 - 20 =		X \$ 18.00	\$			
Independent Claims	5 - 3 =	2	X \$ 80.00	\$160.00			
Multiple dependent claim(s)(if applicable)			+ \$270.00	\$			
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$1,020.00			
Reduction by 1/2 for filing by small entity, if applicable.				- \$510.00			
<b>SUBTOTAL =</b>				\$510.00			
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 month from the earliest claimed priority date (37 CFR 1.492(f)).				\$			
<b>TOTAL NATIONAL FEE =</b>				\$510.00			
				Amount to be refunded	\$		
				Charged	\$		

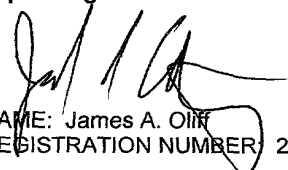
a. ☒ Check No. 115823 in the amount of \$510 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$\_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 15-0461. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:  
 OLIFF & BERRIDGE, PLC  
 P.O. Box 19928  
 Alexandria, Virginia 22320

  
 NAME: James A. Oliff  
 REGISTRATION NUMBER: 27,075  
  
 NAME: Joel S. Armstrong  
 REGISTRATION NUMBER: 36,430

09/744679

525 Rec'd PCT/PTO 29 JAN 2001

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Rachel BAR-SHAVIT

Application No.: U.S. National Stage of  
PCT/IL99/00079

Filed: January 29, 2001

Docket No.: 108366

For: METHOD FOR TREATMENT OF INVASIVE CELLS

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office  
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Please replace claim 18 as follows:

18. (Amended) A method according to claim 17 wherein said antisense molecule is  
administrated to a trophoblast cell.

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JAN 29 2001  
PCT/PTO

REMARKS

Claims 1-19 are pending. Claim 18 is amended to eliminate an incorrect dependency.

Prompt and favorable consideration on the merits is respectfully requested.

The attached Appendix includes marked-up copies of each rewritten paragraph (37 C.F.R. 1.121(b)(iii)) and claim (37 C.F.R. 1.121(c)(ii)).

Respectfully submitted,



James A. Oliff  
Registration No. 27,075

Joel S. Armstrong  
Registration No. 36,430

Enclosure:  
Appendix

JAO:JSA/kaf  
Date: January 29, 2001

**OLIFF & BERRIDGE, PLC**  
**P.O. Box 19928**  
**Alexandria, Virginia 22320**  
**Telephone: (703) 836-6400**

DEPOSIT ACCOUNT USE AUTHORIZATION Please grant any extension necessary for entry; Charge any fee due to our Deposit Account No. 15-0461
--

09744679-011101

## APPENDIX

### Changes to Claims:

The following is a marked-up version of the amended claim: 18

18. A method according to claim ~~48~~ 17 wherein said antisense molecule is administrated to a trophoblast cell.

PTO/PST Rec'd 29 AUG 2001

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Rachel BAR-SHAVIT

BOX: SEQUENCE

Application No.: 09/744,679

Filed: April 11, 2001

Docket No.: 108366

For: METHOD FOR TREATMENT OF INVASIVE CELLS

SUPPLEMENTAL PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office  
Washington, D. C. 20231

Sir:

In reply to the Notification of a Defective Response mailed May 21, 2001, please  
amend the above-identified application as follows:

IN THE DRAWINGS:

Please replace Figure 10 as set forth in the attached Request for Approval of Drawing  
Correction.

IN THE SPECIFICATION:

At the end of the application, please insert the attached paper and computer-readable  
Sequence Listing.

Page 5, lines 14-19, delete current paragraphs and insert therefor:

- 1) The protease activated domains and hirudin binding domain:

Nucleotides

hPAR-1(ThR) 37-61..... TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 1)

hPAR-2 32-56.....SSKGRSLIGKVDGTSHVTGKGVTVE (SEQ ID NO: 2)

hPAR-3 34-57.....TLPIKTFRGAPPN SFEEFPFSALE (SEQ ID NO: 3)

hPAR-4 28-52.....LPAPRGYPGQVCANDSDTHELPDSS (SEQ ID NO: 4)

Page 6, lines 1-3, delete current paragraphs and insert therefor:

**Fig. 1** shows the DNA (SEQ ID NO: 5) and amino acid sequence (SEQ ID NO: 6) of human ThR [1].

**Fig. 2** shows the DNA sequence of an antisense cDNA of ThR (SEQ ID NO: 7).

**Fig. 3** shows the location of the ThR antisense in the pcDNA III vector.

Page 7, lines 24-26, delete current paragraphs and insert therefor:

**Fig. 9** shows the DNA sequence of PAR-2 (SEQ ID NO: 8).

**Fig. 10** shows the DNA sequence of PAR-3 (SEQ ID NO: 9).

**Fig. 11a** shows the DNA sequence of PAR-4 (SEQ ID NO: 10).

**Fig. 11b** shows the amino acid sequence of PAR-4 (SEQ ID NO: 11).

Page 15, lines 4-20, delete current paragraphs and insert therefor:

To analyze the impact of reduced ThR expression in the highly metastatic cells, MDA-435 breast carcinoma cells were transfected with an antisense ThR cDNA (SEQ ID NO: 7) mammalian expression vector containing ThR cDNA in an antisense orientation under the control of the Cytomegalovirus (CMV) promoter (see Figs. 2 and 3). The vector alone was used as a control. Western blot analysis of ThR protein levels showed a marked reduction in the antisense transfected cells (Fig. 8, lane A) as compared to vector alone (lane B) or untreated MDA-435 cells (lane C). When the antisense transfected cells were tested in the Matrigel invasion assay, the otherwise aggressively invading cells showed a markedly reduced level of invasion, similar to that of the non-metastatic breast carcinoma cell line MCF-7 (Fig. 8, E&F). Transfection with the vector alone had no effect on the invasion

properties and the transfected cells migrated effectively through the Matrigel layer (D), similar to the metastatic MDA-435 cells (A).

Similar antisense molecules may be prepared from other members of the PAR family, such as PAR-2 (SEQ ID NO: 8) (Fig. 9), PAR-3 (SEQ ID NO: 9) (Fig. 10) and PAR-4 (SEQ ID NO: 10) (Fig. 11a).

#### REMARKS

Claims 1-19 are pending. The attached Appendix includes marked-up copies of each rewritten paragraph (37 C.F.R. §1.121(b)(1)(iii)).

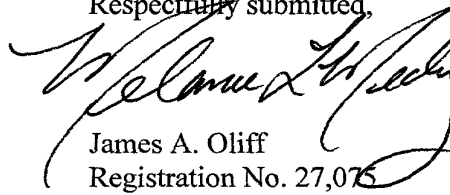
The attached paper copy and computer-readable copy of the Sequence Listing are submitted in compliance with 37 C.F.R. §§1.821-1.825. The contents of the paper copy and the computer-readable copy of the Sequence Listing are the same.

Support for the information provided in the Sequence Listing can be found in the specification at, for example, page 5 and in Figures 1, 2, 9 and 11, and in new Figure 10 filed herewith. Figure 10 is being replaced in order to correct an inadvertent error that was introduced into the specification. In particular, current Figure 10 recites the murine DNA sequence of PAR-3, rather than the human DNA sequence of PAR-3, which was clearly intended. New Figure 10 recites the human DNA sequence of PAR-3. The human DNA sequence of PAR-3 was known in the art at the time the present application was filed. As evidence of this, attached is a printout of Accession No. U92971 of the NCBI database indicating that the human DNA sequence of PAR-3 was known in the art. Thus, including this sequence in Figure 10 does not introduce new matter into the specification. As a result, no new matter is added in the Sequence Listing.



Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,



James A. Oliff

Registration No. 27,075

Melanie L. Mealy

Registration No. 40,085

JAO:MLM/jca

Attachments:

Appendix

NCBI Database Printout

Sequence Listing (paper and computer-readable copies)

Request for Approval of Drawing Correction

Date: August 29, 2001

**OLIFF & BERRIDGE, PLC**  
**P.O. Box 19928**  
**Alexandria, Virginia 22320**  
**Telephone: (703) 836-6400**

**DEPOSIT ACCOUNT USE  
AUTHORIZATION**

Please grant any extension  
necessary for entry;

Charge any fee due to our  
Deposit Account No. 15-0461

## APPENDIX

## Changes to Specification:

A Sequence Listing is added.

Page 5, lines 14-19:

- 1) The protease activated domains and hirudin binding domain:

Nucleotides

hPAR-1(ThR) 37-61..... TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 1)

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hPAR-3 34-57.....TLPIKTFRGAPPN SFEEFPFSALE (SEQ ID NO: 3)

hPAR-4 28-52.....LPAPRGYPGQVCANDSTHELPDSS (SEQ ID NO: 4)

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**Fig. 11b** shows the amino acid sequence of PAR-4 (SEQ ID NO: 11).-and

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To analyze the impact of reduced ThR expression in the highly metastatic cells, MDA-435 breast carcinoma cells were transfected with an antisense ThR cDNA- (SEQ ID NO: 7) mammalian expression vector containing ThR cDNA in an antisense orientation under the control of the Cytomegalovirus (CMV) promoter (see Figs. 2 and 3).

The vector alone was used as a control. Western blot analysis of ThR protein levels showed a marked reduction in the antisense transfected cells (Fig. 8, lane A) as compared to vector alone (lane B) or untreated MDA-435 cells (lane C). When the antisense transfected cells were tested in the Matrigel invasion assay, the otherwise aggressively invading cells showed a markedly reduced level of invasion, similar to that of the non-metastatic breast carcinoma cell line MCF-7 (Fig. 8, E&F). Transfection with the vector alone had no effect on the invasion properties and the transfected cells migrated effectively through the Matrigel layer (D), similar to the metastatic MDA-435 cells (A).

Similar antisense molecules may be prepared from other members of the PAR family, such as PAR-2 (SEQ ID NO: 8) (Fig. 9), PAR-3 (SEQ ID NO: 9) (Fig. 10) and PAR-4 (SEQ ID NO: 10) (Fig. 11a).

525 Rec'd PCT/PTO 29 JAN 2001

**METHOD FOR TREATMENT OF INVASIVE CELLS****FIELD OF THE INVENTION**

This invention relates to the therapeutic use of molecules associated with protease activated receptors.

**BACKGROUND OF THE INVENTION**

5       References referred to by bracketed numbers in the body of the specification are listed at the end of the specification before the claims.

      The process by which epithelial cells become invasive is complex and has yet to be fully elucidated. One example of this process is observed in metastatic tumors. Another example of epithelial cells becoming invasive occurs  
10       during normal human embryonic development, in which the cytotrophoblasts (i.e. the fetal cells on the front line of the placenta) invade the uterus, as part of their normal differentiation program and successful implantation.

      The physiologic invasiveness of cytotrophoblasts closely resembles that of malignant cells, sharing many common features. Tumor invasion and  
15       metastasis involve, among other alterations, proteolytic modification of basement membranes and extracellular matrices (ECMs). Cancer cells have to detach from their primary location, encounter basement membranes (i.e. during extravasation of blood or lymphatic vessels), and disseminate through the circulation to establish new cellular colonies at distant sites. Therefore, the  
20       process of cell invasion involves a well-orchestrated sequence of events including integrin activation, cell migration and proteolytic degradation of specific barrier components. This enzymatic cleavage is highly regulated, since extensive proteolysis could impede the invasive process by degrading essential

matrix components required for the transmission of survival and cell shape signals, through contacts with the basement membrane. Localized proteolysis directed to discrete regions of the cell surface may facilitate cellular invasion.

The thrombin-receptor (ThR) is a seven transmembrane domain  
5 G-coupled protein, belonging to the protease-activated receptor (PAR) family [1]. Recently, two other members of this family (PAR-2 and PAR-3) have been identified [2-4], and a fourth member (PAR-4) has also been described [19]. Unlike most cellular growth factor receptors, the activation of these receptors does not require formation of the traditional ligand-receptor complex. Instead,  
10 the receptor serves as a substrate for proteolytic digestion, yielding an irreversible form of activated cell surface protein to convey further cell signaling.

Applicant's co-pending Israel Patent Application No. 114890, whose contents are incorporated herein by reference, discloses that a direct correlation  
15 exists between ThR level of expression in tumor cells and their degree of invasiveness. This finding was used to develop a diagnostic method for evaluating the metastatic tendency of tumor cells by following the expression of the ThR gene.

U.S. 5,352,664 to Carney, *et al*, describes thrombin-derived polypeptides  
20 which are capable of selectively stimulating or inhibiting thrombin receptor occupancy signals. Carney suggests that the inhibitory polypeptides may be used in preventing metastasis and angiogenesis. No supporting data is disclosed.

## SUMMARY OF THE INVENTION

25 It is an object of the present invention to provide a method for treating metastatic tumors.

It is a further object of the present invention to provide a method for treating irregularities in physiological placental development.

The present invention is based on the surprising finding that interfering with the expression of PAR proteins of an invasive cell affects its degree of invasiveness. The interference may be realized at the DNA (gene) level, at the mRNA level, and/or at the protein (receptor) level. Interference at the DNA level  
5 may be achieved by use of gene therapy methods; interference at the mRNA level may be achieved by use of antisense molecules; and interference at the protein level may be achieved by use of specific antibodies.

The PAR protein may be any member of the PAR family such as, for example but not limited to, ThR, PAR-2, PAR-3 and PAR-4.

10 In a first aspect of the invention, the invasive cells are pathological cells such as metastatic tumor cells. Thus, in this aspect of the invention, there is provided a method for treating metastatic tumor cells of a subject comprising administering to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence  
15 of a PAR protein.

Also provided are antisense molecules and pharmaceutical compositions comprising them.

Further provided is a method for treating metastatic tumor cells of a subject comprising administering to said subject an antibody molecule, said antibody  
20 molecule being capable of binding to a protease activated receptor (PAR) protein. The antibody molecule may be a polyclonal or monoclonal antibody, prepared by methods known *per se*.

In this aspect of the invention, the tumor cells will generally be of epithelial origin, which form solid carcinoma-type tumors. Examples of such epithelial  
25 tissues are breast, esophagus, kidney, prostate, ovary, melanoma and bladder tissue.

In a second aspect of the invention, the invasive cells are normal cells such as placental cells. As described above, ThR plays a role during cytotrophoblast invasion and implantation. The finding that ThR expression is  
30 associated with the invasiveness of placental tissue may be beneficial for

improved implantation of human embryo in the maternal uterus decidua. To date, the rate of spontaneous abortions is 8-12%, 50% of which are due to defects in proper implantation. It is even more striking in the I.V.F. procedure, where 40% of the overall cases result in failure. 90% of these failures are  
5 apparently due to implantation defects. Transfection of normal placenta with ThR and other PAR family genes may considerably improve implantation.

Thus, in this aspect of the invention, there is provided a method for the treatment of disorders involving the implantation of a placenta in a female subject comprising administering to said subject an antisense molecule, said antisense  
10 molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a PAR protein.

Also provided are antisense molecules and pharmaceutical compositions comprising them.

The synthesis of antisense molecules to known mRNA sequences is well  
15 known to the skilled artisan. In theory, based on Watson-Crick base pair formation, if an appropriate target can be identified, an antisense oligomer of more than 15 to 17 nucleotides in length would be expected to have a unique sequence relative to the entire human genome. A suitable oligomer should be able to interfere, in a sequence specific manner with the process of mRNA  
20 translation into protein [9]. The requirements for an antisense oligomer for therapeutic use are: (1) that it must be stable *in vivo*; (2) it must be able to enter the target cell; and (3) it must be able to interact with its cellular targets.

As oligomers possess little or no innate ability to diffuse across cell membranes, the cells must take them up through energy-dependent mechanisms.  
25 To resolve the problem of uptake, a large number of strategies have been employed in order to augment the rate of cellular internalization of nucleic acids and to increase the rate at which they pass through the endosomal membrane. These strategies include: (i) coupling oligomers to polycations such as polylysine [10], polyethylamine [11] or others; (ii) use of  
30 transferin/polylysine-conjugated DNA in the presence of the capsid of a

replication-deficient adenovirus [12]; (iii) conjugation of oligonucleotides to fusogenic peptides [13] or to a peptide fragment of the homeodomain of the *Drosophila* antennapedia protein [14]; (iv) targeting of oligonucleotides to specific cell surface receptors, such as folate, asialoglycoprotein receptor and transferrin [15], (v) conjugation to cholesterol [16]; and, most successfully (vi) complexation of oligonucleotides with cationic lipids [17] and GS 288 etofectin [18].

Preferred antisense sequences are those designed to comprise sequences which hybridize to uniquely conserved regions in the PAR family of proteins. Conserved regions may be identified by comparing the nucleotide sequences of different members of the PAR family. For example, certain regions within the ThR sequence have 27% sequence similarity to PAR-3 and 28% similarity to PAR-2. Examples of conserved unique regions are:

1) The protease activated domains and hirudin binding domain:

15	<u>Nucleotides</u>
hPAR-1(ThR)	37-61..... TLDP <u>RSFLLRNPNDKYEPFWEDEEK</u>
hPAR-2	32-56.....SSKGR <u>SLIGKVDGTSHVTGKGVTVE</u>
hPAR-3	34-57.....TLPIK <u>TFRGAPPN</u> <u>SFEEFPFSALE</u>
hPAR-4	28-52.....LPAPR <u>GYPGQVCANDSDTHELPDSS</u>

2) Second extracellular loop: located between transmembrane domains 4 & 5 and corresponding to residues: **ITTCHDV** which are conserved in PAR 1-3, while in PAR-4 only the three amino acids **CHD** are conserved.

3) The entire promoter region of the PAR family (i.e. 5' cloned regions downstream to the ATG of PAR-1 and PAR-3). This region is likely to contain important regulatory sequences.

#### DETAILED DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:



Fig. 1 shows the DNA and amino acid sequence of human ThR [1];

Fig. 2 shows the DNA sequence of an antisense cDNA of ThR;

Fig. 3 shows the location of the ThR antisense in the pcDNA III vector;

Fig. 4 illustrates ThR expression in human breast carcinoma cell lines.

- 5 Total RNA isolated from human breast carcinoma cell-lines was analyzed by Northern blotting. The cell lines used were: MDA-435 (A), MDA-231 (B) and MCF-7 (C), as well as Ha-ras-transfected breast carcinoma cell lines, MCF10AT3B (D), MCF10AT (E) and MCF10A (F). The blots were probed with <sup>32</sup>P-labeled 250 base pair DNA, corresponding to ThR (upper part), or
- 10 with <sup>32</sup>P-labeled  $\beta$ -actin DNA (lower part).

- Fig. 5 illustrates immunocytochemical analysis of cell-associated ThR. Human breast carcinoma cell lines (MCF-7, MDA-231, and MDA-435) were cultured in 8-well chamber slides and analyzed for the presence of ThR. Specific staining of the receptor was obtained following incubation with affinity
- 15 purified polyclonal anti ThR antiserum followed by biotin conjugated goat-anti-rabbit IgG antibodies and detected by extravidin incubation. Photographs of representative areas of MCF-7 (a), MDA-231 (b) and MDA-435 (c) cell monolayers are shown (x400).

- Lower Panel.** Western blot analysis of ThR. Western blot analysis of cell
- 20 lysates (50 $\mu$ g/lane) of MCF-7 (A), MDA-231(B) and MDA-435 (C) cells. Specific protein band was detected following incubation with anti ThR antibodies and visualized by the ECL immunoblotting detection system according to the manufacturer's instructions.

- Fig. 6 illustrates *in situ* hybridization of ThR mRNA in normal and
- 25 cancerous breast tissue specimens. Hybridization with ThR riboprobes was performed on: Normal breast duct lobular units (A&D). Invasive duct carcinoma, (IDC) (antisense orientation, C; sense orientation, B). High grade DCIS of comedo type (antisense orientation, E; sense orientation, F). Low grade DCIS, solid type (G) and atypical intraductal hyperplasia (AIDH, H & I).

Detection of specifically hybridized mRNA to DIG-labeled probe was performed using anti-DIG-alkaline phosphatase conjugated antibodies (Boehringer Mannheim, Mannheim, Germany). These analyses represent at least 3 patients of each category.

5       **Fig. 7** illustrates Matrigel invasion of breast carcinoma cell lines. The indicated cells (ZR-75, A; MCF-7, B; MDA-435, C; MDA-231, D; fibrocystic MCF10AT3B, E; fibrocystic MCF10A, F) were applied ( $2 \times 10^5$  cells/assay) to the upper compartment of Boyden chambers. Cell invasion through Matrigel coated filters was determined, as outlined in Materials and Methods, below.

10       **Fig. 8** illustrates inhibition of MDA-435 Matrigel invasion by ThR antisense. MDA-435 cells were transiently transfected with pCDNAIII expression plasmid containing the antisense ThR of Fig. 2. The level of invasion was compared to untreated MDA-435 (A) and MCF-7 (B) cells. Control transfections of MDA-435 cells were performed in the presence of vector alone  
15 - (C) or DOTAP liposomes alone (Gibco -BRL) (D). Nearly confluent (60%) cells were treated with various concentrations of the plasmid: transfection with antisense ThR - 5  $\mu$ g/plate (E), transfection with antisense ThR - 20  $\mu$ g/plate (F). The invasion assay was performed as described under Materials and Methods, 72 h following transfection.

20       **Lower panel.** Western blot analysis of ThR antisense transfectants. MDA-435 cell lysates (50 $\mu$ g/lane) of ThR antisense transfectants (A) were applied on SDS-PAGE and the level of receptor protein was compared to cells transfected with vector alone (B) or untreated cells (C).

**Fig. 9** shows the DNA sequence of PAR-2;

25       **Fig. 10** shows the DNA sequence of PAR-3;

**Fig. 11** shows the DNA sequence of PAR-4; and

**Fig. 12** illustrates expression of ThR in first trimester human placenta. *In situ* hybridization analysis of ThR expression at 6-15 weeks of gestation. Placental tissue was obtained from elective termination of pregnancies by  
30 dilatation and curettage. Sections of 6 week placental tissue (A) and of 7, 8, 9

and 10 weeks of gestation (B-E, respectively), as visualized by ThR staining of cytotrophoblasts. No staining was observed at weeks 11 and 15 (F & G, respectively). Control hybridization (weeks 7 and 8) using sense orientation showed background staining (H & I, respectively).

5

## DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

### Materials and Methods

**Cells:** The human breast carcinoma cell lines, MCF-7 (adenocarcinoma), MDA-MB-231 (adenocarcinoma), MDA-MB-435 (ductal carcinoma) and ZR-75-1 (carcinoma), were kindly provided by Dr. Robert Stern (Department of Pathology, University of California, San Francisco). The invasive properties of these breast cell lines were determined following injection of the cells into the mammary pads of nude mice with or without Matrigel [5]. Cells were cultured in DMEM (1g glucose/liter) containing 10% bovine calf serum. MCF10A (nearly-normal immortalized epithelial cells), MCF10AT cells (derived from human fibrocystic epithelium transfected with Ha-ras) and MCF10AT3B cells (derived from a 94-day third transplant generation of lesion in Beige /Nude mice, classified as grade 2), were kindly provided by Dr. F. R. Miller (Karamanos Cancer Institute, Meyer L. Prentiss Center, Detroit) and grown in RPMI-1640 containing 10% fetal calf serum (FCS). Tissue culture medium was supplemented with penicillin (50 U/ml) and streptomycin (50 µg/ml) (GIBCO-BRL, Gaithersburg, MD) and the cells were maintained at 37°C in a 10% CO<sub>2</sub> humidified incubator. Cells were dissociated with 0.05% trypsin/0.02% EDTA, 0.01M sodium phosphate (pH 7.4) solution (STV) and subcultured at a split ratio of 1:5.

**Plasmids and transfection:** The DNA and amino acid sequences of ThR are shown in Fig. 1 [1]. ThR in the antisense orientation (Fig. 2), consisting of 612 nucleotides (from (-)75 to (+)537 of Fig. 1) was prepared and inserted into the

eukaryotic expression plasmid, pcDNA III (Invitrogen, Carlsbad, CA) at the HindIII and EcoRI sites (Fig. 3). Antisense ThR cDNA was used for transient transfection experiments. Subconfluent (25-40%) MDA-435 breast cancer cells were grown in 60 mm culture dishes and a total of 5-20 µg of DNA and DOTAP - transfection reagent (10 µg DOTAP/µg DNA; 4.5 h incubation, Boehringer Mannheim, Mannheim, Germany) were used for transfection. Cells were assayed 48-72 h following transfection.

**RNA Isolation and Northern blot analysis:** RNA was prepared using TRI-Reagent (Molecular Research Center, Inc. Cincinnati) according to manufacturer's instructions. The RNA (20 µg of total RNA) was separated by electrophoresis through a 1.1% agarose gel containing 2 M formaldehyde, transferred to a nylon membrane (Hybond N<sup>+</sup>; Amersham) and hybridized either to cDNA probes or PCR product radiolabeled by random primer extension with [ $\alpha$ -<sup>32</sup>P]Dct [6] for 24 h at 42°C. The membrane was washed twice for 30 min at room temperature with 2x SSC containing 2% SDS and 15 min at 50°C with 0.1x SSC, containing 0.1% SDS. The blots were exposed for 2-4 d at -70°C and the relative amounts of mRNA transcripts were analyzed by laser densitometry using an Ultrosan XL Enhanced Laser Densitometer and normalized relative to internal  $\beta$ -actin controls.

**In situ hybridization of human tumor and placenta biopsy specimens.** RNA probes were transcribed and labeled by T<sub>7</sub> RNA polymerase (for antisense orientation) or T<sub>3</sub> RNA polymerase (for sense control orientation) using DIG-UTP labeling mix (Boehringer Mannheim, Mannheim, Germany). Probes were labeled from plasmid containing 462 base pair fragments of the human ThR (pBhThR-462S) inserted into the EcoRI-HindIII site. Final concentration for hybridization was 1 µg/ml, according to the manufacturer's instructions for non radioactive *in situ* hybridization application. Hybridization was carried out

(overnight, 45°C) on paraffin embedded breast tissue sections (Department of Pathology, Hadassah University Hospital, Jerusalem) or placenta sequential sections. Slides were washed in 0.2xSSPE (3x 1 h) at 50°C and blocked by blocking reagent (Boehringer Mannheim, Mannheim, Germany). Detection was performed using AP-conjugated, anti-DIG antibodies (Fab-fragment, diluted 1:300; 5  
Boehringer Mannheim, Mannheim, Germany), overnight at room temperature. AP reaction was detected by NBT/BCIP reagents according to the manufacturer's instructions.

- 10 **Immunohistochemistry:** Tumor cells were cultured overnight at 37°C on eight chamber slides. The cells were fixed with 2% formaldehyde and 2% sucrose/PBS at room temperature for 30 min and permeabilized with 20 mM Hepes, pH 7.4, 300 mM sucrose, 50 mM NaCl, 3 mM MgCl<sub>2</sub> and 0.5% Triton X-100, for 4 min at 0°C. After rehydration with PBS, the cells were incubated  
15 (10 min, 24°C) with 3% H<sub>2</sub>O<sub>2</sub> in PBS containing 10 mM glycine, 10 mg/ml BSA, followed by 30 min blocking with normal goat serum in PBS containing 1% BSA. Affinity purified rabbit-anti-human ThR antibodies were added (dilution 1:50-1:200) for 4 h at 4°C, followed by incubation (1 h, room temperature) with a second antibody goat-anti-rabbit IgG-Biotin conjugated and  
20 1 h incubation with HRP-ExtraAvidin (1:200) (Sigma Immuno Chemicals, St. Louis, MO).

**Antibodies:** We have raised anti-ThR antibodies directed toward a synthetic peptide (thrombin- receptor activating peptide; TRAP) corresponding to  
25 residues Ser42-Lys51 (i.e. S-F-L-L-R-N-P-N-D-K). KLH conjugated peptide was injected to rabbits, and the immune serum was affinity purified. ELISA was performed on plates coated with the TRAP-peptide showing efficient positive identification at 1:25,600 dilution. Maximal response was obtained at 1:3,200

dilution. Monoclonal anti ThR Abs (mouse IgG1 clone IIaR-A) were used for Western blot analysis (Biodesign, ME, USA)

**Western blotting analysis:** Cells were dissolved in lysis buffer containing 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and protease inhibitors (5 µg/ml aprotinin, 1µM phenylmethylsulfonylfluoride and 10 µg/ml leupeptin) for 30 min at 4°C. After centrifugation at 10,000 g for 20 min at 4°C, the supernatants were transferred and the protein content was measured. Lysates (50 µg ) were loaded and resolved on 10% SDS-PAGE followed by transfer to Immobilon-P membrane (Millipore, MA). Membranes were blocked and probed with anti-ThR antibodies (1:4000) in 1% BSA in 10 mM Tris-HCl (pH 7.5), 100 mM NaCl and 0.05% Tween-20). After washes, blots were incubated with the appropriate second antibodies and conjugated to horseradish peroxidase. Immunoreactive bands were detected by the enhanced chemiluminescence (ECL) reagent using luminol and p-cumaric acid (Sigma, St. Louis, Mo).

**Placental tissue sections:** Sections of placental tissue, 6-15 weeks of gestation, were obtained from elective termination of normal pregnancies by dilatation and curettage.

**Matrigel invasion assay:** Blind well chemotaxis chambers with 13 mm diameter filters were used for this assay. Polyvinylpyrrolidone-free polycarbonate filters, 8 µm pore size (Costar Scientific Co., Cambridge, MA), were coated with basement membrane Matrigel (25 µg/filter) as previously described [7]. Briefly, the Matrigel was diluted to the desired final concentration with cold, distilled water, applied to the filters, dried under a hood, and reconstituted with serum-free medium. Cells ( $2-3 \times 10^5$ ), suspended in DMEM containing 0.1% bovine serum albumin were added to the upper chamber.

Conditioned medium of 3T3 fibroblasts was applied as a chemoattractant and placed in the lower compartment of the Boyden chamber. Assays were carried out at 37°C in 5% CO<sub>2</sub>. Over 90% of the cells attached to the filter after a 2h incubation. At the end of the incubation, the cells on the upper surface of the filter were removed by wiping with a cotton swab. The filters were fixed in methanol and stained with hematoxylin and eosin. Cells in various areas of the lower surface were counted and each assay was performed in triplicate. For chemotaxis studies, filters were coated with collagen type IV alone (5µg/filter) to promote cell adhesion. Cells were added to the upper chamber and conditioned medium was applied to the lower compartment.

## Examples

### Example I: ThR expression in breast carcinoma cell lines.

In a preliminary experiment, a panel of mammary carcinoma cells was surveyed for a possible correlation between the level of ThR expression and established degrees of metastasis (Fig. 4). The cell lines used included one near-normal diploid immortalized breast epithelial cell line (MCF10A) originating from fibrocystic disease, and 6 tumor cell lines exhibiting different doubling times, tumorigenicity and metastases in nude mice. Of these cell lines, MDA-435 (a highly metastatic cell line), and MCF10AT3B (ras transfected fibrocystic epithelium re-established several times from lesions formed in nude mice), were compared to medium metastatic (MDA-231 and MCF10AT, ras transfected fibrocystic cells), or carcinoma cells exhibiting no metastatic potential (ZR-75 and MCF-7 cells). As shown in Fig. 4, high levels of ThR mRNA were found in the highly aggressive cells (lanes A, D) as compared to moderate levels in MDA-231 and ras transfected fibrocystic cells (lanes B& E, respectively), and no expression in the non-metastatic MCF-7 and MCF10AT cells (lanes C&F, respectively). The mRNA levels were quantified by densitometric analysis and the ratio of ThR/β-actin in each lane was calculated. The ThR mRNA level in MDA-435 was 6 fold higher than in MDA-231 cells

(Fig. 4, lanes A vs B) and, as mentioned above, no detectable ThR was observed in MCF-7 cells (Fig. 4, lane C). A similar correlation between ThR level of expression and metastasis was obtained in Ha-ras transfected cells showing a 4 fold higher level in MCF10AT3B (obtained following ras-transfection and xenografting 3 times in mice) than in MCF10AT-ras transfected cells (Fig. 4, lanes D vs E). No detectable level of expression was observed in the fibrocystic, non-malignant, epithelial cells, MCF10A epithelial cells (Fig. 4, lane F).

Affinity purified rabbit-anti-human ThR antibodies were applied to detect the expression and localization of the receptor protein. Massive staining of MDA-231 and MDA-435 cells was observed (Fig. 5B&C, respectively), as opposed to little or no staining of MCF-7 cells (Fig. 5A). In parallel, Western blot analysis showed a distinct protein band of ThR in MDA-435 cells (Fig. 5, lower panel; lane C), somewhat reduced ThR level in MDA-231 (lower panel; lane B) and little or no protein in MCF-7 breast carcinoma cells (lower panel; lane A).

Collectively, these data demonstrate the preferential expression of ThR in metastatic breast carcinoma cell lines, but not in non-metastatic MCF-7 or MCF10A breast carcinoma cells, regardless of whether the mRNA or protein levels were evaluated.

#### **Example 2: ThR expression in human breast tissue specimens.**

ThR gene expression and localization *in vivo* was studied in formalin fixed paraffin embedded human breast carcinoma specimens as compared to normal mammary sections obtained from reduction mammoplasty. ThR expression was examined in primary breast tumors representing poor to benign prognosis. *In situ* hybridization analysis using a ThR RNA probe (corresponding to nucleotide nos. 320-570 of the sequence of Fig. 1) was performed with an archival set of paraffin embedded biopsy specimens. A total of 10 normal breast tissue specimens, and 8 specimens of infiltrating ductal carcinoma were analyzed. The invasive carcinoma specimens were selected



from typical infiltrating duct carcinoma of high nuclear grade with numerous atypical mitotic figures and with evidence of vascular invasion and lymph node metastases.

As demonstrated in Fig. 6, hybridization of a ThR antisense RNA probe to invasive duct carcinoma specimens resulted in strong positive staining localized specifically to the carcinoma cells (Fig. 6C). Weaker positive staining was noted in high-grade ductal carcinoma *in situ* (DCIS) of comedo-type (Fig. 6 E&F). In contrast, very little or no staining was observed in low-grade, solid type DCIS (Fig. 6G), and no staining was observed in premalignant atypical intraductal hyperplasia (AIDH) (Fig. 6 H&I) and in normal breast duct lobular units (Fig. 6 A&D; note that the high staining seen in the background is limited to the fibers, and is not seen in the epithelial cells). AIDH was distinguished from low grade DCIS, non-comedo type according to the diagnostic criteria of Dupont, Page and Rogers [8]. Expression was also noted in some cases of DCIS, in particular, high grade, comedo-type lesions. The low grade DCIS of solid type showed weak to no expression of ThR, while cases of AIDH, as well as normal breast tissue from reduction mammoplasty specimens did not show any expression of ThR.

**Example 3: Antisense ThR inhibits metastatic breast carcinoma cell invasion.**

To assess the invasion properties of aggressively metastatic breast carcinoma cells, the Matrigel *in vitro* invasion assay was applied. For this purpose, a reconstituted matrix of basement membrane was utilized to coat porous filters, in order to closely mimic natural barriers in a Boyden chamber. As a chemoattractant source, fibroblast conditioned medium was placed in the lower compartment [7]. The Matrigel invasion assay confirmed the expected differential metastatic properties of the carcinoma cell lines. High levels of invasion through Matrigel were obtained with MDA-435 and MDA-231 cells (Fig. 7, D&C). MCF10AT3B-ras transfected fibrocystic cells invaded the

Matrigel to a lower extent (Fig. 7, E), while no movement was detected with the MCF10AT, MCF-7, or ZR-75 non-metastatic cell lines (Fig. 7, F & A ,B, respectively).

To analyze the impact of reduced ThR expression in the highly metastatic  
5 cells, MDA-435 breast carcinoma cells were transfected with an antisense ThR  
cDNA. mammalian expression vector containing ThR cDNA in an antisense  
orientation under the control of the Cytomegalovirus (CMV) promoter (see  
Figs. 2 and 3). The vector alone was used as a control. Western blot analysis of  
ThR protein levels showed a marked reduction in the antisense transfected cells  
10 (Fig. 8, lane A) as compared to vector alone (lane B) or untreated MDA-435  
cells (lane C). When the antisense transfected cells were tested in the Matrigel  
invasion assay, the otherwise aggressively invading cells showed a markedly  
reduced level of invasion, similar to that of the non-metastatic breast carcinoma  
cell line MCF-7 (Fig. 8, E&F). Transfection with the vector alone had no effect  
15 on the invasion properties and the transfected cells migrated effectively through  
the Matrigel layer (D), similar to the metastatic MDA-435 cells (A).

Similar antisense molecules may be prepared from other members of the  
PAR family, such as PAR-2 (Fig. 9), PAR-3 (Fig. 10) and PAR-4 (Fig. 11).

20

#### **Example 4: ThR expression during placenta development.**

Human embryo development depends on proper placentation and  
successful implantation. Trophoblast invasion through the uterine epithelium  
and deep into the stroma enables the establishment of the proper fetal-maternal  
25 interactions. Histological examination of placental biopsies during the first  
trimester (6-15 weeks), obtained from elective termination of pregnancies,  
showed a striking pattern of ThR temporal regulation. ThR mRNA levels were  
not detected up to 6 weeks of gestation (Fig. 12,A), increased markedly between  
7-10 weeks (B-E), then fell precipitously at 11 weeks and thereafter (F&G).  
30 The staining was specific to ThR, since hybridization with ThR sense

orientation on placental biopsies taken on weeks 7 and 8, showed no staining (H&I, respectively). The receptor appeared localized to the cytotrophoblasts within the villi, and also, to some extent, in the syncytiotrophoblasts of the invading column.

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**CLAIMS:**

1. A method for treating metastatic tumor cells of a subject comprising administering to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence  
5 of a protease activated receptor (PAR) protein.
2. A method according to claim 1 wherein said PAR protein is a thrombin receptor.
3. A method according to claim 1 wherein said PAR protein is selected from the group consisting of PAR-2, PAR-3 and PAR-4.
- 10 4. A method according to claim 1 wherein said tumor cell is of epithelial tissue origin.
5. A method according to claim 4 wherein said epithelial tissue is selected from the group consisting of breast, esophagus, kidney, prostate, ovary, melanoma and bladder.
- 15 6. A method according to claim 1 wherein said antisense molecule has the sequence appearing in Fig. 2.
7. A method for treating metastatic tumor cells of a subject comprising administering to said subject an antibody molecule, said antibody molecule being capable of binding to a protease activated receptor (PAR) protein.
- 20 8. A method according to claim 7 wherein said antibody binds an extracellular epitope of said PAR protein.
9. An antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.
- 25 10. A pharmaceutical composition comprising an active factor and a pharmaceutically acceptable carrier, said active factor being an antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

11. A pharmaceutical composition according to claim 10 for the treatment of metastatic tumor cells.

12. A pharmaceutical composition according to claim 11 wherein said PAR protein is a thrombin receptor.

5 13. A pharmaceutical composition according to claim 11 wherein said PAR protein is selected from the group consisting of PAR-2, PAR-3 and PAR-4.

14. A pharmaceutical composition according to claim 11 wherein said tumor cell is of epithelial tissue origin.

10 15. A pharmaceutical composition according to claim 14 wherein said epithelial tissue is selected from the group consisting of breast, esophagus, kidney, prostate, ovary, melanoma and bladder.

16. A pharmaceutical composition according to claim 10 wherein said antisense molecule has the sequence appearing in Fig. 2.

15 17. A method for the treatment of disorders involving the implantation of a placenta in a female subject comprising administering to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

20 18. A method according to claim 18 wherein said antisense molecule is administered to a trophoblast cell.

19. A pharmaceutical composition according to claim 10 for the treatment of disorders involving the implantation of a placenta in a female subject.

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1  ggcgcgcgc  gacgcgcgc  ccagtcgcc  cccgcgccg  ctaaccgcc  cagacacagc
61  gctgcgcgag  ggtcgcttgg  acctgatct  taccgtggg  caccctgcg  tctgcctgcc
121  gcgaagaccg  gctccccgac  cgcagaagt  caggagagag  ggtgaagcgg  agcagcccca
181  ggcggggcag  cctccccgag  cagcgcgcg  cagagcccg  gacaatggg  ccgcggcgcc
241  tgctgctggt  ggcgcgcgc  ttcagtctg  ggcggccgc  gttgtctgc  cgcacccggg
301  cccgcaggcc  agaatcaaaa  gcaacaaatg  ccaccttaga  tcccgggtca  tttcttctca
361  ggaaccccaa  tgataaatat  gaaccatttt  gggaggatga  ggagaaaaat  gaaagtgggt
421  taactgaata  cagattagtc  tccatcaata  aaagcagtc  tcttcaaaaa  caacttctg
481  catctatctc  agaagatgcc  tccgatat  tgaccagctc  ctggctgaca  ctcttctcc
541  catctgtgta  caccggagt  tttgtagtca  gcctccact  aaacatcatg  gccatcgtg
601  tgttcacct  gaaatgaag  gtcaagaagc  cggcggtgg  gtacatgctg  cacctggcca
661  cggcagatgt  gctgttctg  tctgtgctc  cctttaagat  cagctattac  tttccggca
721  gtgattggca  gtttgggtct  gaattgtgc  gcttcgtcac  tgcagcattt  tactgtaaca
781  tgtacgcctc  tatcttgctc  atgacagtca  taagcattga  ccggttctg  gctgtgggtg
841  atcccatgca  gtccctctcc  tggcgtactc  tgggaaggcg  ttccttact  tgtctggcca
901  tctgggcttt  ggccatcgca  ggggtagtgc  ctctcgtcct  caaggagcaa  accatccagg
961  tgcccgggct  caacatcact  acctgtcatg  atgtgctcaa  tgaaccctg  ctgaaaggct
1021  actatgccta  ctacttctca  gccttctctg  ctgtcttctt  ttttgtgccg  ctgatactt
1081  ccacggctctg  ttatgtgtct  atcattcgat  gtcttagctc  ttcgcagatt  gccaacgca
1141  gcaagaagtc  ccgggctttg  ttcctgtcag  ctgctgtttt  ctgcatcttc  atcattgct
1201  tcggacccac  aaacgtcctc  ctgattggcg  attactcatt  ccttctcac  acttccacca
1261  cagaggctgc  ctacttgcc  tacctcctct  gtgtctgtgt  cagcagcata  agctcgtgca
1321  tcgacccct  aattactat  tacgttct  ctgagtggca  gaggtacgtc  tacagtatct

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**Fig. 1a**

1381 tatgctgcaa agaaagtcc gatcccagca gttataacag cagtgggcag ttgatggcaa  
 1441 gtaaaatgga tacctgtctct agtaacctga ataacagcat atacaaaaag ctgttaactt  
 1501 aggaaaaggg actgctggga ggttaaaaag aaaagtatat aaaagtgaat aacctgagga  
 1561 ttctattagt cccacccaa actttattga ttcacctctt aaaacaacag atgtacgact  
 1621 tgcatacctg ctthttatgg gagctgtcaa gcatgtattt ttgtcaatta ccagaaagat  
 1681 aacaggacga gatgacgggtg ttattccaag ggaatatatgc caatgctaca gtaataaatg  
 1741 aatgtcactt ctggatatag ctaggtgaca tatacactat tacatgtgtg tataatgtaga  
 1801 tgtatgcaca cacatatatt atttgcagtg cagtatagaa taggcacttt aaacactct  
 1861 ttccccgcac ccagcaatt atgaaaaataa tctctgattc cctgatttaa tatgcaaagt  
 1921 ctagggttggg agagtttagc cctgaacatt tcatgggtgtt catcaacagt gagagactcc  
 1981 atagtttggg ctgtgaccac ttttgcaaat aagtgtattt tgaaattgtt tgacggcaag  
 2041 gtttaagtta ttaagaggta agacttagta ctatctgtgc gtagaagttc tagtgttttc  
 2101 aattttaaac atatccaagt ttgaattcct aaaattatgg aaacagatga aagcctctg  
 2161 ttttgatatg gtagtatatt ttacattttt acacactgta cacataagcc aaactgagc  
 2221 ataagtcctc tagtgaatgt aggtggcctt tcagagtagg ctattcctga gagctgcatg  
 2281 tgtccgcccc cgatggagga ctccaggcag cagacacatg ccaggggccat gtcagacaca  
 2341 gattggccag aaacctcct gctgagcctc acagcagtga gactggggcc actacatttg  
 2401 ctccatcctc ctgggattgg ctgtgaactg atcatgttta tgagaaactg gcaaaagcaga  
 2461 atgtgatata ctaggaggta atgaccatga aagacttctc taccatctt aaaaacaacg  
 2521 aaagaaggca tggacttctg gatgcccatc cactgggtgt aaacacatct agtagttgtt  
 2581 ctgaaatgtc agttctgata tggaagcacc cattatgcgc tgtggccact ccaataggtg  
 2641 ctgagtgtac agagtggaaat aagacagaga cctggccctca agagcaaaagt agatcatgca

Fig. 1a (Cont.)



2701 tagagtgtga tgtatgtgta ataaatatgt ttcacacaaa caaggcctgt cagctaaaga  
 2761 agtttgaaca tttgggttac tatttcttgt ggttataact taatgaaac aatgcagtac  
 2821 aggacataata ttttttaaaa taagtctgat ttaattgggc actatttatt tacaatgtt  
 2881 ttgctcaata gattgctcaa atcaggtttt cttttaagaa tcaatcatgt cagtctgctt  
 2941 agaaataaca gaagaaaata gaattgacat tgaaatctag gaaaattatt ctataatttc  
 3001 catttactta agacttaatg agacttttaa agcatttttt aacctcctaa gtatcaagta  
 3061 tagaaaatct tcatggaatt cacaaaagta ttgggaaatt aggtgaaac atatctctta  
 3121 tcttacgaaa aatggtagc attttaaca aaatagaaag ttgcaaggca aatgtttatt  
 3181 taaaagagca ggccaggcgc ggtggctcac gcctgtaac ccagcacttt gggaggctga  
 3241 ggcggttga tcacgaggtc aggagatcga gaccatcctg gctaacacgg tgaaccccg  
 3301 ctctactaaa aatgcaaaaa aatagccg ggcgtggtgg caggcacctg tagtcccagc  
 3361 tactcgggag gctgaggcag gagactggcg tgaacccagg aggcggacct tgtagtgagc  
 3421 cgagatcgcg ccaactgtgct ccagcctggg caacagagca agactccatc tcaaaaaaaa

Fig. 1a (Cont.)

MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLD  
PRSFLLRNPNDKYEPFWEDEEEKNESGLTEYRLVSINKSS  
PLQKQLPAFISEDASGYLTSSWLTFLVPSVYTGVFVWSL  
PLNIMAIVVFILKMKVKKPAVVYMLHLATADVLFVSVLPFK  
ISYYFSGSDWQFGSELCRFVTA AFYCNMYASILLMTVISI  
DRFLAVVYPMQSLSWRTLGRASFTCLAIWALAIAGVVPL  
VLKEQTIQVPGLNITTCHDVLNETLLEGYYAYYFSAFSAV  
FFFVPLIISTVCYVSIIRCLSSSAVANRSKKSRAFLSAAV  
FCIFIICFGPTNVLLIAHYSFLSHTSTTEAAYFAYLLCVCVS  
SISSCIDPLIYYYASSECCQRYVYSILCCKESSDPSSYNSS  
GQLMASKMDTCSSNLNNSIYKKLLTZ

Fig. 1b

5'-CGCCGAGGGTCGCTTGGACCTGATCTTACCCGTGGGCACCCCTGGGCCTCTGCTGCCC  
 GCGAAGACCGGCTCCCCGACCCGCAGAAATCAGGAGAGAGGGTGAAGCGGAGCAGCCCCGA  
 GCGGGGCAGCCTCCCGGAGCAGCGCCCGCAGAGCCCGGGACAAATGGGGCCCGGGGGC  
 TGCTGTGTGGCCGCTGCTTCACTGTGTGGGGCCCGCTGTTGTCTGCCCCGACCCCGGG  
 CCCGAGGCCAGAAATCAAAAGCAACAATGCCACCTTAGATCCCCCGGTCAATTTCTTCTCA  
 GGAAACCCCAATGATAAATATGAACCAATTTTGGGAGGATGAGGAGAGAAAAATGAAAGTGGGT  
 TAACTGATACAGATTAGTCTCCATCAATAAAAGCAGTCCTCTTCAAAAACAACCTTCCTG  
 CATTCACTCAGAAAGATGCCCTCCGGATAATTTGACCAGCTCCTGGCTGACACTCTTTGTCC  
 CATCTGTGTACACCGGAGTGTGTGTAGTCAGCCTCCCACTAAACATCATGGCCCATCGTTG  
 TGTTTCATCCTG-3'

Fig. 2

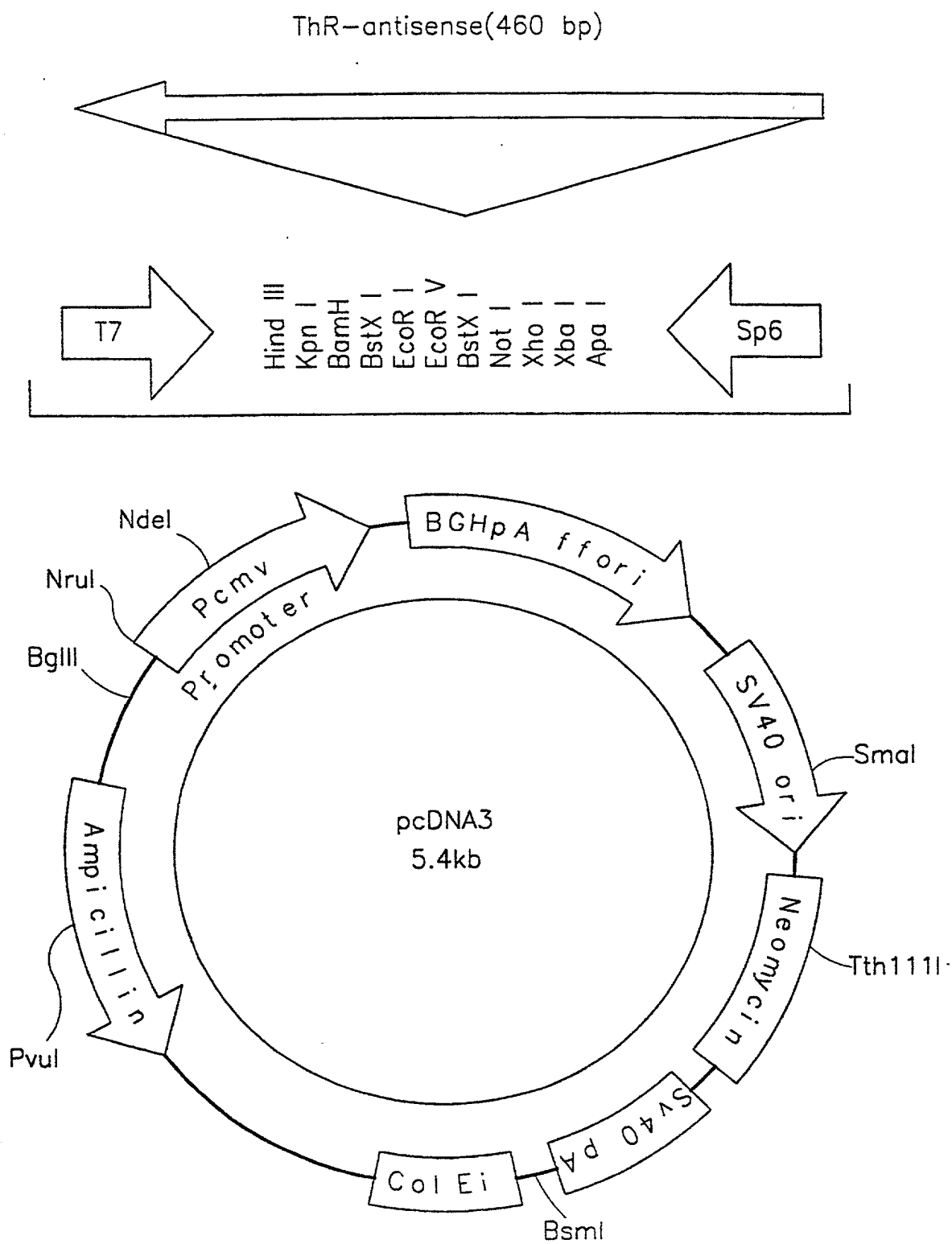


FIG.3

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FIG.4

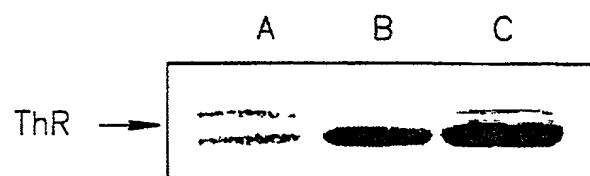
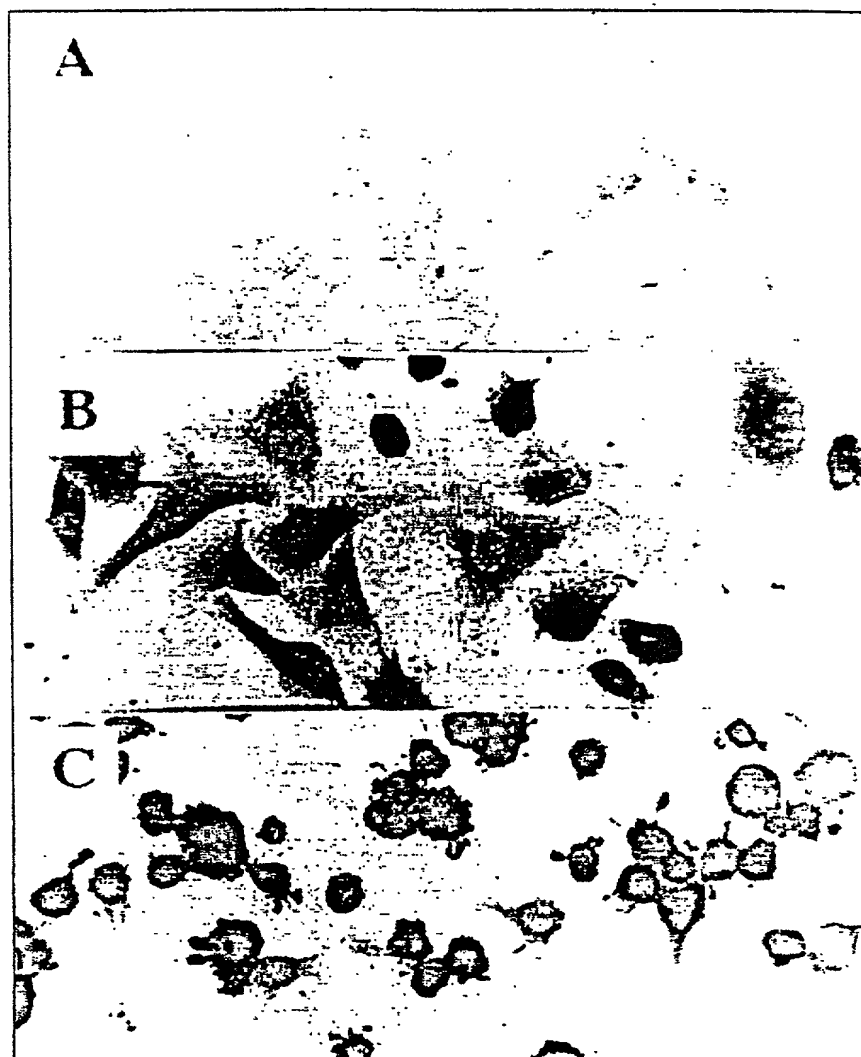


FIG.5

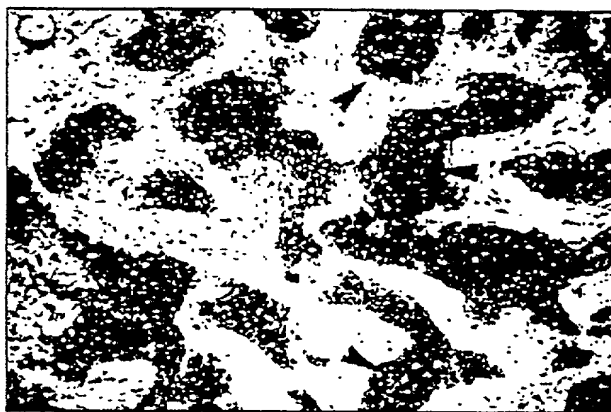


FIG.6C

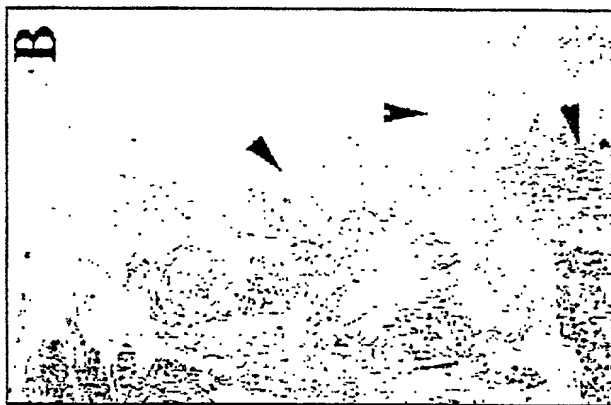


FIG.6B

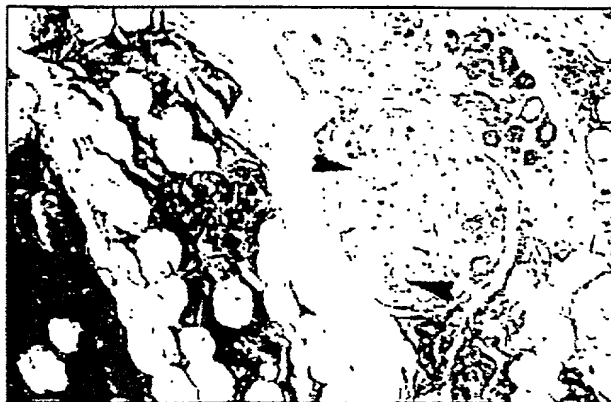


FIG.6A

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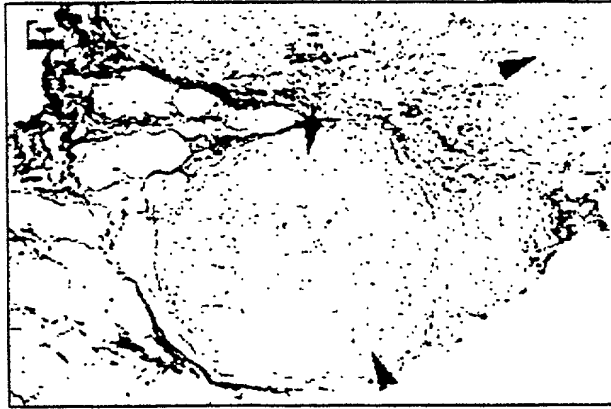


FIG. 6F



FIG. 6E

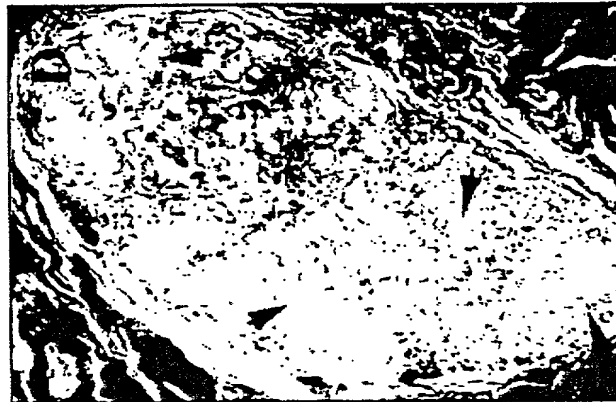


FIG. 6D





FIG. 6I

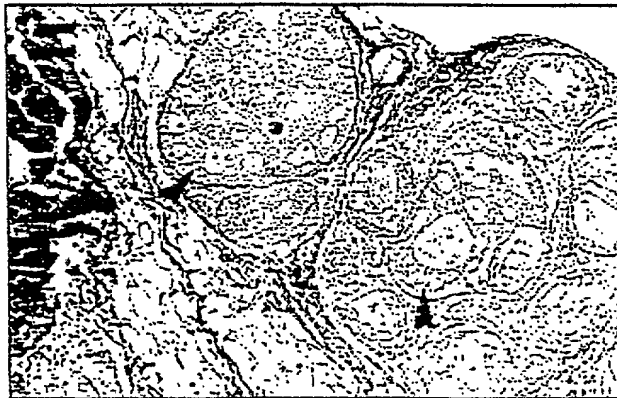


FIG. 6H

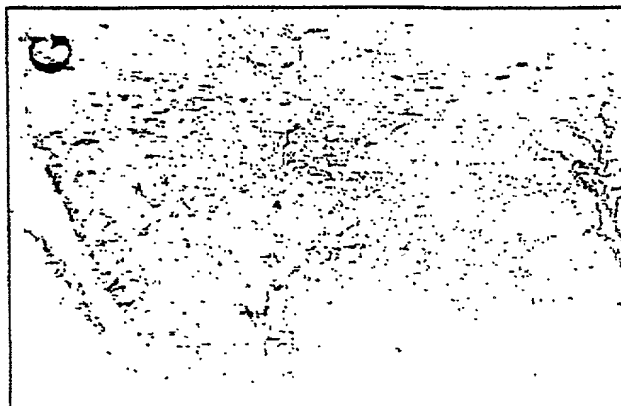


FIG. 6G

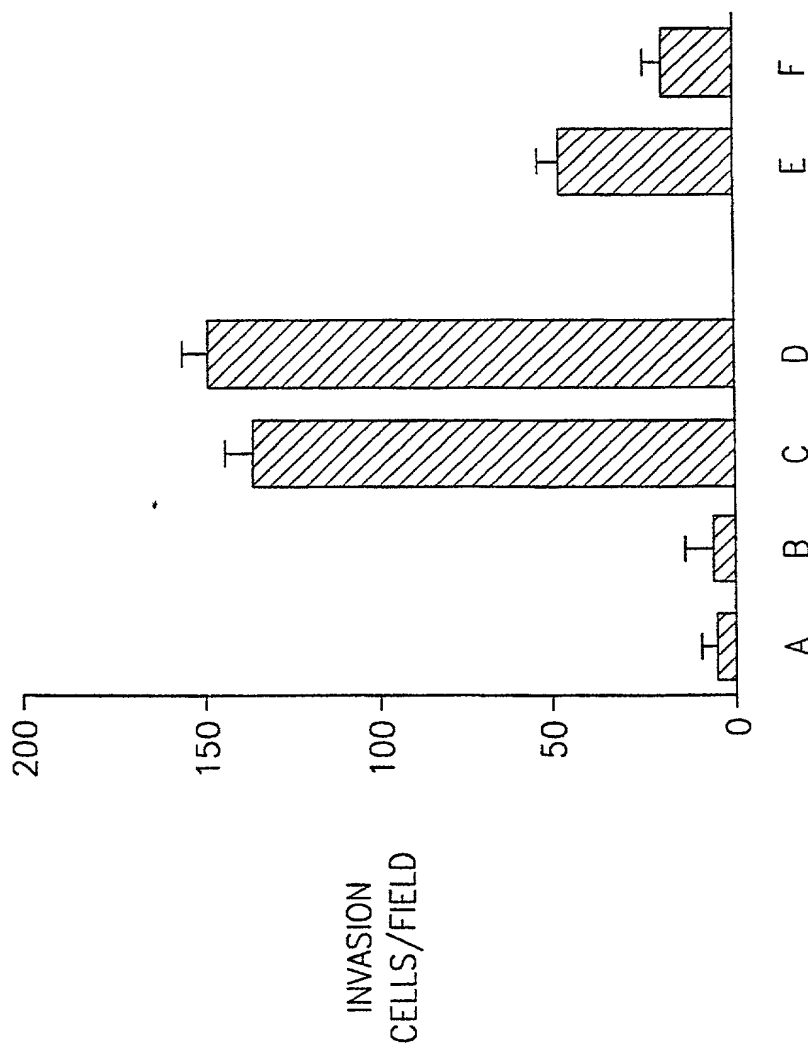


FIG.7

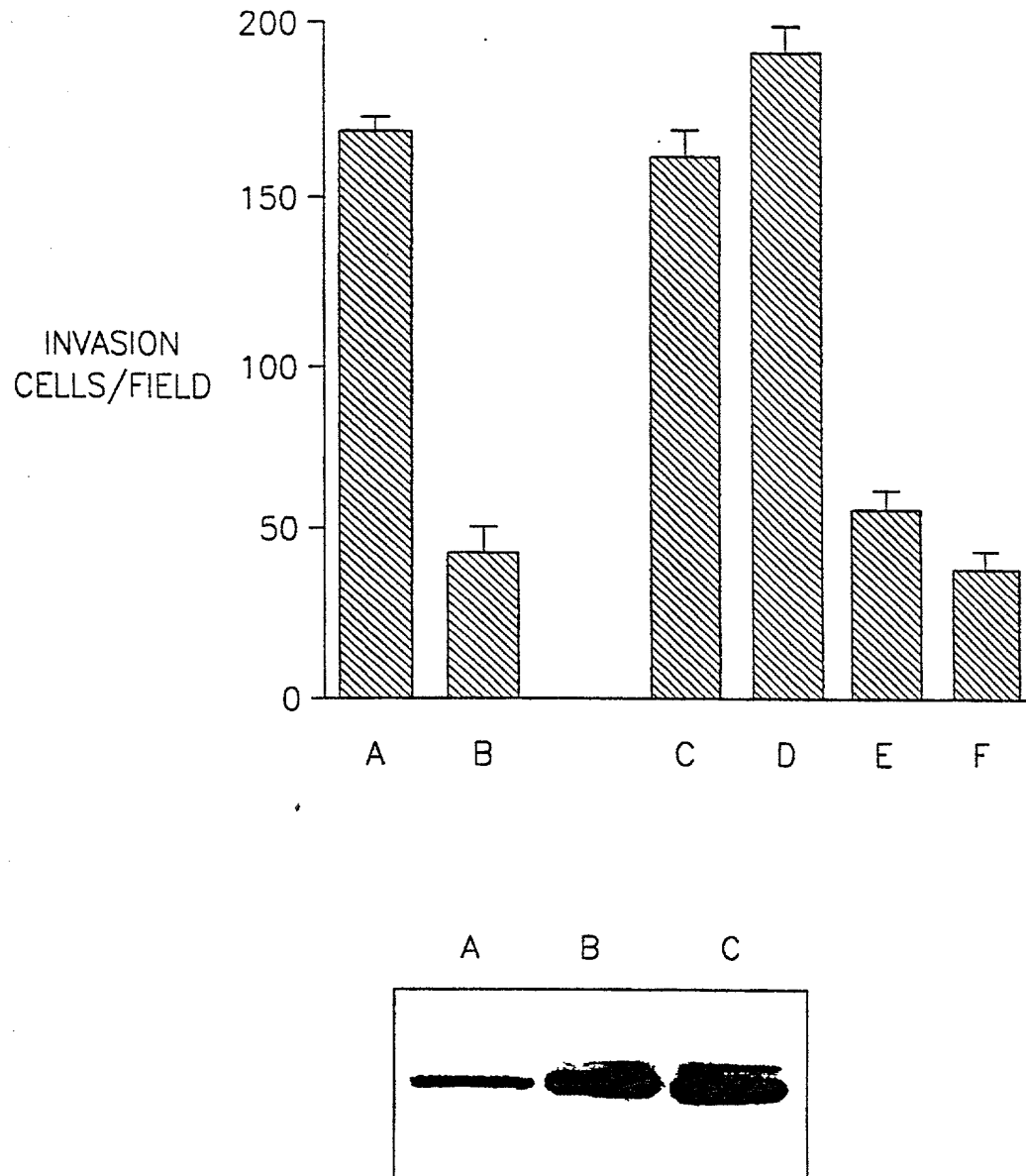


FIG.8

1 a a a t g a a t a a a t g a a t g t a c t t t g a a c a a c c a g t g t t a c t g c t g a a c a t t a  
 61 t t t c t g t a a t g a c c c t t g t c t t c c t t t c t t g a c a g g a a c c a t a g a t c c t a a a g g a a  
 121 g a a g c c t t a t t g g t a a g g t t g a t g g c a c a t c c a c g t c a c t g g a a a a a c g g c a t g g  
 181 a a a c a g t c t t t t c t g t g g a t a c a a t t g t g t c g a g t t t c t g a a g a g c a c c t a c a t g g c c a  
 241 t c t t c c t t c c c t t c t t t c c g a a c t a a g a t c t g t t c c c t t g a a g a t t g t g c c a t a c a  
 301 c c c t g t g g g t g g t g a c c t c t c t c t g t c a t a t t g t a a t g t g c t t a t t g t t t t c t  
 361 a t c t g g c c t t c a a c t g g a t t a t t g g g g a a g a g a c c t g c c t a g a a g g c a a a g c a g a c c a  
 421 t a c a t g g c a a g t a c t g t t c c a t t c t c t c a c a c c a g g a c a t c c c t t t g t a t t g c a g  
 481 a t g g c a a c a t c c c a t g g g g c t g c t g a t t a t c a c g a c c t t g g c c a t t g g g a g g c a t c t c c c  
 541 t c a t c g t g a a g c t g c t g a t t t g c c c t g a a c a t c a g a c c t t c a g a t c t c t c t g g a g a g c a g a c c a  
 601 t g g c a a t a t g t g c c t g a a c c t c t g a t t g t g c c a t g a t t t g g c c a t t g g g c c a g a c c t c c c  
 661 t c t t c a t t c c g t t c a a t t a c t t c c t c t c t c t c t c a a a c t g a t t g t g c c c a g c c t  
 721 t g g g a g a c a t g t t c a a t t a c t t c c t c t c t c t c t c a a a c t g a t t g t g c c a t t g g g c c c a g c c t  
 781 t c c t c a c a g c c t c t g c c t a t g a g a g a a g g a a g a g g g c c a t a g a a c t c a t t g c t c a c t g t c  
 841 a a a c t c a g a a a c t c a t t c a c t c c t a g a c c c t t c t a t t a c t t a t g c c c t g t a t g c c a t g t  
 901 a c c t g a t c t g g a g c c a t g t c g a c c c c t t c c t t t g c c g a a g t g t c c g c a t c c a g a t g g g  
 961 g c c a g g g c c a g a g c c a t g t c g a c c c c t t c g a c c c c t t t a t g c c c t g t a t g c c a t g c a a  
 1021 a c a g c t g c a t c c t t t g c c g a c t c c a g g a a c t c c a g c t c t t a a t t g c a c a g a t g g g  
 1081 a g a a c g c t c t c c t a t t g a g t g t c c g c a t c c a g a t g g g a a t t g c a c a g a t g g g  
 1141 c a a g a a a c a t t t c c a g g t c g a c g t g t c t g a c g a t g g g a a t t g c a c a g t a g g a c c t  
 1201 c c t a t t g a g t t t c c a g g t c t c a g a t g g g t a g g a t g t g g t a a c c t g t t a  
 1261 a t g t t a t g a g a c g t g t c t g a c g a t g g g a a t t g c a c a g t a g g a t g t g g a a c c t g t t a

Fig. 9

1 cggcagcagc aaggacgagt ccctgcccac acagtccagg ctggcagagt tctcagottt  
 61 ccacttgcgt ctcatacatg gagctgaggg gaatctaccc tgggactttg tatacttaac  
 121 aacatcctgt agccgggtct caggacatca agatgaaaat ccttatcttg gttgcagctg  
 181 ggtgctgttt tctgccagtc actgtttgcc aaagtggcat aaatgtttca gacaactcag  
 241 caaagccaac ctttaactatt aagagtttta atgggggtcc ccaaaaatacc tttgaagaat  
 301 tcccactttc tgacatagag ggctggacag gagccaccac aactataaaa gcggagtgtc  
 361 ccgaggacag tatttcaact ctccacgtga ataatgctac cataggatac ctgagaagtt  
 421 ccttaagtac ccaagtgata cctgccatct atatcctgct gtttgtggtt ggtgtacct  
 481 ccaacatcgt gaccctgtgg aaactctcct taaggaccaa atccatcagt ctggtcatct  
 541 ttcacacca cctggccatc gcagatctcc ttttctgtgt cacactgcca tttgaagatc  
 601 cctaccatct caatggcaac aactgggtat ttggcgaggt catgtgccgg atcaccacgg  
 661 tcgttttcta cggcaacatg tactgcgcta tctgtatcct cacttgcatg ggcatacaac  
 721 gctacctggc cacggctcac cttttcacat accagaagct gcccaaacgc agottctcct  
 781 tgctcatgtg tggcatagtg tgggtcatgg ttttcttata catgctgccc tttgtcatcc  
 841 tgaagcagga gtaccacctc gtccactcag agatcaccac ctgccacgat gtgctcgacg  
 901 cgtgcgagtc cccatcatcc ttccgattct actactcgt ctcccttagca ttctttgagt  
  
 961 tctcatccc gtttgtgato atcatcttct gttacacgac tctcatccac aaacttaaat  
 1021 caaaggatcg gatattggtg ggctacatca aggcctgctt cctcatcctt gtgattttca  
 1081 caatttgctt tgccccacc aacatcatac tctgaatcca ccatgccaac tactactacc  
 1141 acaataccga cagcttgtag tttatgtatc ttattgctct gtgcctgggg agcctgaata  
 1201 gctgcctaga tccattcctt tactttgtca tgtcgaaaag tgtagatcag cttaatcctt  
 1261 agtcggcaat ggcaagacca ctttagagac caaggagaga tatctgggaa gacatacatg  
 1321 cttggctgac ttatgcatgg caccaacagc tcaattttta attttaattt aattttatct  
 1381 ttttgagaca gaacctcact gtgtagtctt ggctggcctg gctgattctc tatttagacc  
 1441 aggttagcct tgaactcaca gagatctgcc tgcttctgcc tcccaagtgc tgggttcaac  
 1501 caggtctggc aagcgtccca tttgtcagct cctctgcaac agtgctttat acttccaatg  
 1561 tgaagtcatg aggattaaaa gaatactttg tatttagaga gtgtcttaat tgtaagcaac  
 1621 attagcagta ttccgatggc aatggacgca tttctatttg ccttctgat tctccaagct  
 1681 atacatgtcc ccttccctcc tgtgagcggg gtccgcagac tgggaggttg tcaactaccg  
 1741 tggttctccc tctcctcaa gcaggatatcc tgtctttggt gtgtctctca ctggggctcc  
 1801 gtgttcatgc cacctctgtc caaagatggc agcctcactg ccagccgagc ctcatttcca  
 1861 cagattgggt taaccttccc tgaacagttt ctgaatacat catgcaatac ttcaactgac  
 1921 cctgcagtc tctgaaatg acaagccctt gcatggcttt cctgggtggag ctgtgcctgc  
 1981 gattccacac accttcacac cgactgtctt cctcccatc cctgtcgctc cactgaaacc  
 2041 ccttctagca tctttgcttt agcctgtcca gcttttcaag tctgttaaag cccattcttc  
 2101 ccttattctc aaactaaaaa catcattttc tttatctgga tttgtaaagc actttcttat  
 2161 ctttaaggca cttctagaca aacaagcctg ctaagctcat ttgcatatag atattacttc  
 2221 cataaggaaat taagttctct gtgtggctcag ctctgtatcc ctgcaagttt catacctgta  
 2281 gattcaacca accacagacc caaaaacctt gtaataataa atataataa ataaaaaaa  
 2341 gttctatttt tcattactcc ntaataataa ntgataataa atataataa ataaaaaaa  
 2401 aaaaaaaaaa

Fig 10

FIG. 11a

1 ctcccacggg ctggctggca agcgccctg gtgggtctgc gggggcagg ggcagccttc  
 61 tggtttatct ccacggcgc gatctgctcg tcgcctcgg cccagaagc tggggctcag  
 121 ggtccggcga ggcaggaagc ctgagggcac agccagagc agcctgagtg cagtcagtg  
 181 ggggcgactg ctctgtggc cctgtgtgt ggggttcagc ctgtctggcg gcaccagac  
 241 cccagcgtc tacgacgaga gcgggagcac cggagggtgt gatgacagca cggcccaat  
 301 cctgcctgcc cccgcggct accaggcca agtctgtgcc aatgacagtg acaccctgga  
 361 gctcccgac agctcacggg cadtcttct gggctgggtg cccaccaggc tggtgccgc  
 421 cctctatggg ctggtccttg tggtagggct gccggccaat gggctggcg tgtgggtgct  
 481 ggcacgcag gaacctcggc tgcctccac catgtgctg atgaacctcg cgactgcga  
 541 cctctgtctg gccctggcg tgcctcccg gatgcctac cactgctg gccagcgctg  
 601 gccctcggg gaggcgct gccgcctgg cagggcgca ctctatggtc acatgtatgg  
 661 ctacgtctg ctgctggcg ccgtcagcct ggtcgtctac ctggccctgg tgcacccgct  
 721 gcgggccgc gccctgctg gccggcgct ggccttga ctctgcatgg ctgcttggct  
 781 catggcgcc gccctggcac tgccttgac actgcagcg cagacctcc ggcggcgcg  
 841 ctccgatgc gtgctctgcc atgacgcgt gccctggac gcacaggcct cccactgct  
 901 accggcctc acctgctgg cgctgtggg ctgttctcg cccctgctgg ccatgctgct  
 961 gtgctacgg gccacctgc acacgctgg gccagcgcc cggcgctacg gccacgcgt  
 1021 gaggtgacc gcagtgggtc tggcctcgc cgtggccttc ttcgtgcca gcaacctgct  
 1081 gctgctgctg cattactcg accgagccc cagcgctgg ggcaacctct atggtgccta  
 1141 cgtgccagc ctggcgctga gcacctca gactgctg gatcccttca tctactata

Fig. 11a

1201 cgtgtcggcc gagttcaggg acaaggtgcg ggcagggtc ttccaacggt cgccggggga  
 1261 caccgtggcc tccaaggcct ctgcggaagg gggcagcgg ggcatgggca cccactcctc  
 1321 ttgtctccag tgacacaaag tggggaaggc tgtactgggt cgaacagggt cccctccccc  
 1381 acttcacgtc cttccigggga cctcagaatg tgacctatt tgaaatatagg gttgttaciaa  
 1441 ctgtcactag cggaggtcac ttggagaag ggtgggcctt acatccagtg tgggtgggtg  
 1501 cctcataaga taaggagagg ccaggccctg tggctcacgc ctgtaatccc agcacitfaa  
 1561 gaggccaagg cggatggatc acttgagccc aggagttcaa caccagcctg agcaacatgg  
 1621 taaaaccca tctctacca aaatacaaaa attagctggg ctgtgtggct ggcgcctgta  
 1681 atcccagcta ctcaggagac tgaggcagaa ggalcgttg aacctgggag gcagagggtg  
 1741 cagtgaagg agattgcgcc actggacicc agcctgcgtg acagagagcc tgtctctaaa  
 1801 ttaattaatt aattaatta attcaattt aaaagacga aaagtgacgg ccagggtgcag  
 1861 tggctcacgc ctataatcic agcacctcgg gaggccaaga tgaggagattg ctgaaagcca  
 1921 ggagtttgg accagcctgg gcaacatagg gggatcccat ctctacacac aaaaaaattt  
 1981 ttaataaac caggcatgtt ggcattgcgc tatagtccca gccactcaag aggcacaggc  
 2041 gggaggatca ctgagccctg ggagggttg gttgcagtga gctatgattg taccactgca  
 2101 ctccagcctg ggcaacagag caagaccctg tctcaaaaat aaacaaacta aaattaaaaa  
 2161 aagaagacga gagatagtgg gtgtgtgtggc tcacaccctg aatccagca ctttgggaagg  
 2221 ccgagggtgg cagatcatct gagggccagga gttaagacc agcctggcta acatggtgaa

Fig. 11a (Cont.)

2281 atcctatctc taccaaaaat acaaaaatta gccaggcgtg gtggtgggca cctgtactgg  
 2341 ggaggtgccc accagctac tggggaggct ggtcaggag aatcgctga accctgggagg  
 2401 cggaggttgc ggtcagctga gatgtgcca ctgcactcca gcttgggca aagagcgact  
 2461 ctgtctcaa aaaaagaga agaggagagg acacagagac acacagagaa gaaagccatg  
 2521 tggcggcaga ggcagagatg ggagtatgc ggacggacac aaactaagg atgccacgat  
 2581 gccaagcaca gccaacagcc accagcagcc aggagacagg cctgggacgg gctctccctc  
 2641 acagcctcca gagggaacca gccctgccac cacttgacc ctggactct ggcttgacga  
 2701 actgtgagac aataaactct catgttita agctgctgg catgtggcac ttgtcaggg  
 2761 cagcccagga atctgaaca ggaalcaact ctgcttctg ggccctgcca gcatctctgg  
 2821 ctgggttctc tgggctggat gcagccacg acgcactggt gtctgagatg gggctggagc  
 2881 tggggctggg gctgcattcc ctggagactc actgcaagt cctgcccagg aggtgaggg  
 2941 caccatcc tcagtgccca atgctgtggc ccaccaggc ccagagcctg gtggccatt  
 3001 ctcatgcca ccagctctg gccttggat gtctgtgag caaccagaat agcaccacca  
 3061 actctgtccc ccaaaaccca tcatagcac ggctcagcct cctgctatcc cctgactgct  
 3121 ggggaccctc gccttcccct cctcaccctg caggctgac ctcttttca ctctgtca  
 3181 atgtcaccag ggataagggt ggacaatggg ggggtgggggt ggacagtgtg tgcggggggg  
 3241 ttgggtgtgt gcagacctgg aactcccttc tgcaggatg ttggcagccg gtgtgaagcc  
 3301 ttgcacggga cagaccacac ccacggcaac ctcatccctc cagcactaac cacatccact

Fig. 11a (Cont.)



3361 ctcaaccccg tcccctcgc actgaccaca ccaccccggt tggccccgc ccccgccact  
 3421 gaacactccc gccctcaacc ccgacccctc cgcactcacc tcccctcgc cgctcgaccc  
 3481 cgccctcacc aactgacca ccctcaacc attgcgcca gtccccacca cagtaccac  
 3541 accctcactg gctcgccct gcccccagla tactgaccat tcccagcca ctcccttc  
 3601 gcacttacca ctccccagc cagccccc cccgctgacc gctcctccag cccgcctcc  
 3661 cccgtacagg cagagcgccc gccacctct atgtgcgtt ctctgactt tacgttgccc  
 3721 cctcctcgc caagcccca ggggagccct cctggcgtc cgagggtggg agtcgggggtg  
 3781 tggcaggccg cgggtggggg cggcagtggc tccgcgact caccggggcc ccgggcaggg  
 3841 gcgcgctcca ctctgttga cgcgggtccg gcgcacagtt cccgggagag tgggctgtgc  
 3901 gtgctgacgt ttagaagcg agtggcctcg aaggctacgg gacgaggggtg gcgggtgacc  
 3961 aagtgcaggc gcgacgggtc agggaccggg ccgggcccgg ggtgcggggc gcggggccta  
 4021 ccgggttcgt agtagtcga caggagact ggcagcgccg acgtcctgccc caccacgcac  
 4081 tcccgagag caggaaaccg caccacgic aggcaccggc tggggatctg tggggcagcg  
 4141 gcgggagcag gctcgacccg ggcagaggagg ccggggggcg tgagctcagg ccagaaactg  
 4201 gctgattca gggatacca ggacgcgtga aacacagaag aaacgtgac ccatttctt  
 4261 tttttcttt acttttctt tttttttt ttctgagac agagtctcg gctgttgccc  
 4321 aggcaggagt gcagtggcgt gatctggct cactgcaagc tcggccctct gggttcaaat  
 4381 gattctctg cctcagcctc ccaagtagct gggataacag gcgcccacca ccgacccctg

Fig. 11a (Cont.)

4441 ctaattttt gtattttga tcaagacgga gtttcacat gttggccagg ctggtctcca  
4501 actcctgccc tcaagtgatc cgcctcggtc ccattttta ttcttgggt ccttccatcc  
4561 cactgggaaa acgtctcagg tggcctctga aacaccactc ctttttgt gtgtgcacgc  
4621 atggctgagc atgtgtgggt gggagtcagc acattcacga tactgtgcaa tcatcacctc  
4681 tgtctagta caggacggtt tcttttccc ccaagaaac ccatcgcca tcagcactca  
4741 ctcccactc cccagcccc tggcaaccac aaatcttcc aactctagg attgacctgt  
4801 tctgggcatt tcatgtcaat ggaatcatgt actctgtgaa aaaaaaaaaa aaaaaaaa  
4861 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa

**Fig. 11a (Cont.)**

MWGRLLWPLVLGFSLSGGTQTPSVYDESGSTGGDDSTPSILP  
 APRGYPGQVCANDSDTLELPDSSRALLLGWVPTRLVPALYGLVLVVGLPANGALWVL  
 ATQAPRLPSTMLLMNLATADLLALALPPRIAYHLRGQRWPFGEAACRLATAALYGHM  
 YGSVLLAAVSLDRYLALVHPLRARALRGRRLALGLCMAAWLMAAALALPLTLQRQTF  
 RLARSDRVLCHDALPLDAQASHWQPAFTCLALLGCFLPLLAMLLCYGATLHTLAASGR  
 RYGHALRLTAVVLASAVAFFVPSNLLLLHYSDPSPSAWGNLYGAYVPSLALSTLNSC  
 VDPFIYYVSAEFRDKVRAGLFQRSPGDTVASKASAEGSGRMGTHSSLLQ

Fig. 11b



FIG. 12C



FIG. 12B

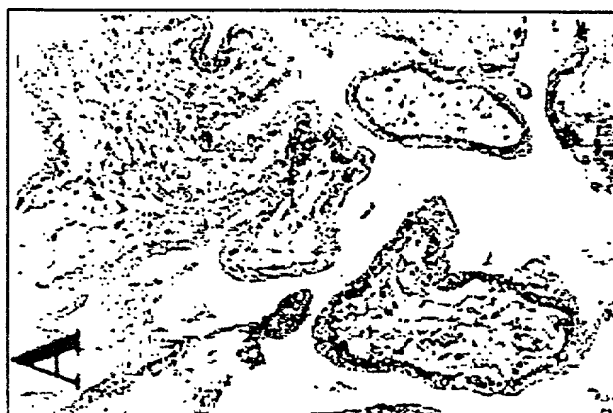


FIG. 12A

FIG. 12D



FIG. 12D

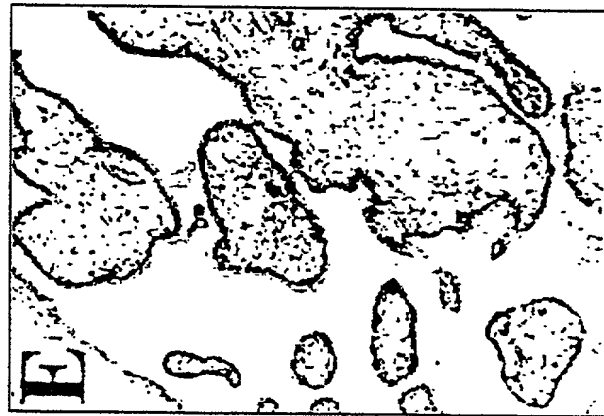


FIG. 12E

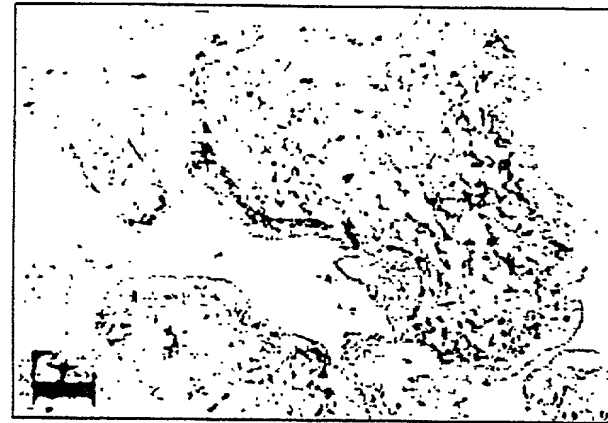


FIG. 12F

FIG. 12G

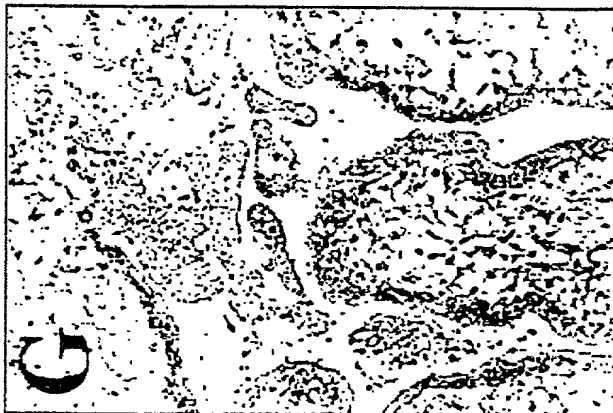


FIG. 12G

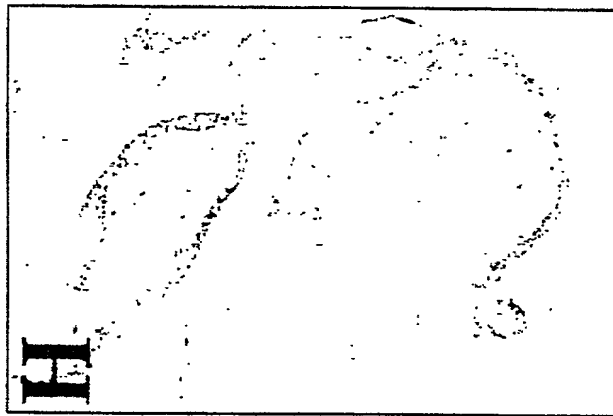


FIG. 12H

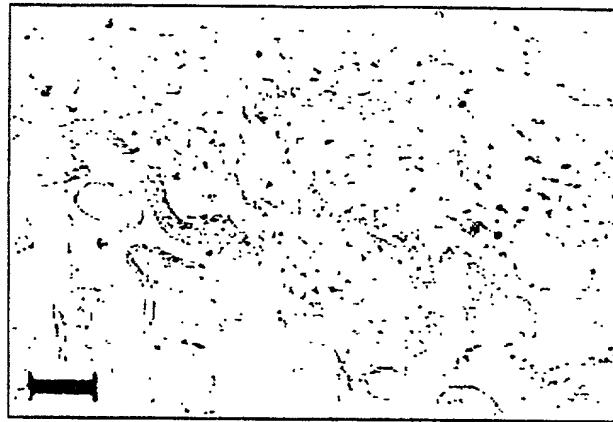


FIG. 12I

FROM :

REINHOLD COHN &amp; PARM

FAX NO. :

972 3 7109407

972 3 7109407

03/21 '01 11:24 Mar. 22 2001 12:05PM P2  
NO. 415 05/04

Docket No.: 108366

# DECLARATION AND POWER OF ATTORNEY UNDER 35 USC §371(c)(4) FOR PCT APPLICATION FOR UNITED STATES PATENT

As a below named inventor, I hereby declare that:  
my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled: METHOD FOR TREATMENT OF INVASIVE CELLS

described and claimed in international application number PCT/IL 99/00079 filed February 5, 1999.

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

Israeli Patent Application No. 125698 filed August 7, 1998.

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,975; William F. Berridge, Reg. No. 36,824;  
Kirk M. Hudson, Reg. No. 27,552; Thomas J. Pardini, Reg. No. 30,411;  
Edward F. Walker, Reg. No. 31,450; Robert A. Miller, Reg. No. 32,771;  
Mario A. Costantino, Reg. No. 33,565; Stephen J. Roe, Reg. No. 34,463;  
Joel S. Armstrong, Reg. No. 36,430; Christopher W. Brown, Reg. No. 38,025; and  
Richard E. Rice, Reg. No. 31,560.

ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1	Typewritten Full Name of Sole or First Inventor	Rachel	BAR-SHAVIT
2	Inventor's Signature	<i>Rachel Bar Shavit</i>	Family Name
3	Date of Signature	March 22	Year 2001
	Residence:	Ramat-Sharet	Israel
	Citizenship:	Israel	Country
	Post Office Address:	44 Zeretetz Bernstein Street	
	(Insert complete mailing address, including country)	Ramat-Sharet Jerusalem 96920 Israel	

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

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05744679 "041101

NCBI! Nucleotide

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1: U92971 Human protease-activated receptor 3 (PAR3) mRNA, complete cds PubMed, Protein, Related Sequences, Taxonomy, OMIM, LinkOut

LOCUS HS092971 1830 bp mRNA PRI 16-APR-1997

DEFINITION Human protease-activated receptor 3 (PAR3) mRNA, complete cds.

ACCESSION U92971

VERSION U92971.1 GI:1938374

KEYWORDS

SOURCE human.

ORGANISM Homo sapiens  
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 1830)

AUTHORS Ishihara, H., Connolly, A.J., Zeng, D., Kahn, M.L., Zheng, Y.W.,  
Timmons, C., Tram, T. and Coughlin, S.R.

TITLE Protease-activated receptor 3 is a second thrombin receptor in  
humans

JOURNAL Nature 386 (6624), 502-506 (1997)

MEDLINE 97242411

REFERENCE 2 (bases 1 to 1830)

AUTHORS Ishihara, H., Connolly, A.J., Zeng, D., Kahn, M.L., Zheng, Y.W.,  
Timmons, C., Tram, T. and Coughlin, S.R.

TITLE Direct Submission

JOURNAL Submitted (11-MAR-1997) CVRI, UCSF, 3rd and Parnassus, San  
Francisco, CA 94143, USA

FEATURES Location/Qualifiers

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CDS 145..1269  
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BASE COUNT 473 a 464 c 337 g 556 t

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